

# Exposure Assessment of Glyphosate in Selected Grains Grown in Gombe State, Nigeria and the Potential Health Risk on Consumption

<sup>1</sup>Zira, S. P., <sup>2</sup>Bwatanglang, I. B., <sup>3</sup>Audu, Haruna Sympa

<sup>1,2</sup>Department of Pure and Applied Chemistry, Adamawa State University, Mubi, Nigeria

<sup>3</sup>Department of Chemistry, Federal College of Education (Technical), Gombe, Nigeria

Corresponding Author's E-mail: [pstorspz@gmail.com](mailto:pstorspz@gmail.com)

**Abstract** - Glyphosate widely accepted to be biodegradable and ecofriendly are arguably found to be ambiguous having pinged its footprints in some environmental and health related complications. This herbicide is used widely in Gombe State, Nigeria to mitigate the growth of transgenic plants, and control weeds infestation in farmlands despite being banned in most countries. Thus present a potential risk to the public that still use the formulations as herbicides. Therefore, in this study, we examined the residual level of glyphosate in the rice, beans, maize, millet and sorghum obtained from Gombe, Kaltungo and Funakaye Local Government Areas (LGA) of Gombe State and further conducted the human health risk assessments on consumption. The samples were purified and subjected to extraction and clean-up procedures using water/acetone (50/50). The results quantified using Gas chromatography coupled with mass spectrophotometer revealed the average concentrations of glyphosate residues in the samples collected from the three LGA to follow the increasing order; 0.03692>0.01501>0.01413 ppm respectively. However, the concentrations were all observed to be lower than the Minimum Residual Limits (MRLs) set by the WHO/FAO. The non-carcinogenic health risk analysis taken from the data, having values of <1, suggest that the residues do not pose any disenable health risk to the public on consumption but further suggest the exposed children and adults populations are at risk of potential carcinogenic-related complications based on the respective measured Life Time Total Cancer Risk Index (TCRI) of  $1.81 \times 10^{-3}$  and  $2.25 \times 10^{-4}$ . The Correlation coefficient established for the samples locations reveals no significant correlation between the pairs of LGAs. From the foregoing results, effective measures and regulations on the use of glyphosate-based herbicides should be instituted.

**Keywords:** Glyphosate Residues, Health Risk, Grains, Herbicides, Staple Food, Gombe State.

## I. INTRODUCTION

Food despite being practically the reservoir of most essential nutrients, also modulates toxicity, inhibits diseases, reduces risk from environmental stressors, and improves life expectancy (Bwatanglang, 2019). However, the deployment of agrochemicals into farming operations has compromised these much needed advantages, steaming largely from the use of herbicides and pesticides which are further reported to leave behind residual footprint across the food chain. To meet food sufficiency amidst the depleting soil vital functions, farmers in Gombe State, Nigeria employed the use of Glyphosate-based herbicide to mitigate the growth of transgenic plants, and control weeds infestation in farmlands without recourse to its potential public health risk. The herbicide (N-phosphonomethyl glycine) is designed to preferentially target transgenic plant (glyphosate herbicide-resistant crops) (JMPR, 2006) and engineered to inhibit/block the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) pathway, the ingredients that stimulates the secretion of phenylalanine, tyrosine and tryptophan essential to plant growth, defense and survival (Pieniazek *et al.*, 2014; Gomes *et al.*, 2015; Aghoghovwia & Izah, 2018). Widely acceptable by farmers; Kruger, *et al.*, 2014, and approved for use in over 130 countries sold under the trade name of Roundup (Pieniazek *et al.*, 2014) and seemingly banned in some countries (Burt, 2019; HBL, 2019). In Nigeria, the National Food and Drug Administration and Control (NAFDAC) enforced a ban on glyphosate-based formulations with tallow-amine (Urowaymo, 2019).

Despite the regulations and enforcement, food profiling conducted by some researchers and regulatory agency, reported traces of glyphosate. Stacy, (2019) reported traces found in most food products in USA, and in bread flour and maize meal in South Africa as reported by Michael, (2019). Glyphosate was also implicated in reported fish death documented from Kano, Nigeria (NAN, 2018) and in non-Hodgkin's lymphoma among French Men (Orsi, *et al.*, 2009; IARC, 2015). The residual levels were also detected in maize

samples taken from Italy, reported by Vincenzo, *et al.*, (2018), in soya beans from Manhattan, USA (Xu, *et al.*, 2019) and in some cereal products from Retail Markets in Canada (Kolakowski, *et al.*, 2020). The residues, even as low as the regulatory limits are reported by Mesnage, *et al.*, (2015) to exhibit carcinogenic-related complications. Further reported by the International Agency for Research on Cancer (IARC) to possess all the characteristic of classical carcinogenic agents (Michael, 2019; Vincenzo, *et al.*, 2018; Stacy, 2019; TPG, 2019).

In Gombe State, Nigeria, farmers struggled to tamed the wake of weeds infestations, observed to outcompete food crops in accessing the already depleted natural nutrients in the over cultivated soils. To mitigate the onslaught of the weeds infestation on the crop performance and their overall yield, the famers resorted on the widespread use of agrochemicals such as glyphosate. Though, no available statistics or data on the quantity of glyphosate applied annually in the study locations, the continual reliance on herbicide to win the war on the weeds infestation further suggest potential health risk to the

farmers on exposure to the chemical. Thus, become imperative for a study to be carried out from the characterization of herbicides residual levels to its potential health risk to the public on consumption of crop cultivated in soil-treated with the herbicides in other to bring more light to the public as relates the deadly incursion of glyphosate to their common wealth, health and food security.

## II. METHODOLOGY

### Description of the Study Area

Gombe State with a land mass of 20265sqm (16,639km<sup>2</sup>) and population of 2.4 million (2,365,040) is located on latitude 9°30' and 12°30'N. Longitude 8°5' and 11°45'E. The state is sharing borders with Borno State in the North-East; Yobe State in the North; Adamawa State in the South East, Taraba State in the South and Bauchi State in the West. The State consist of eleven (11) LGA whose populations are predominantly farmers (Fig 1) (MGS, 2020 and NHDR, 2018).

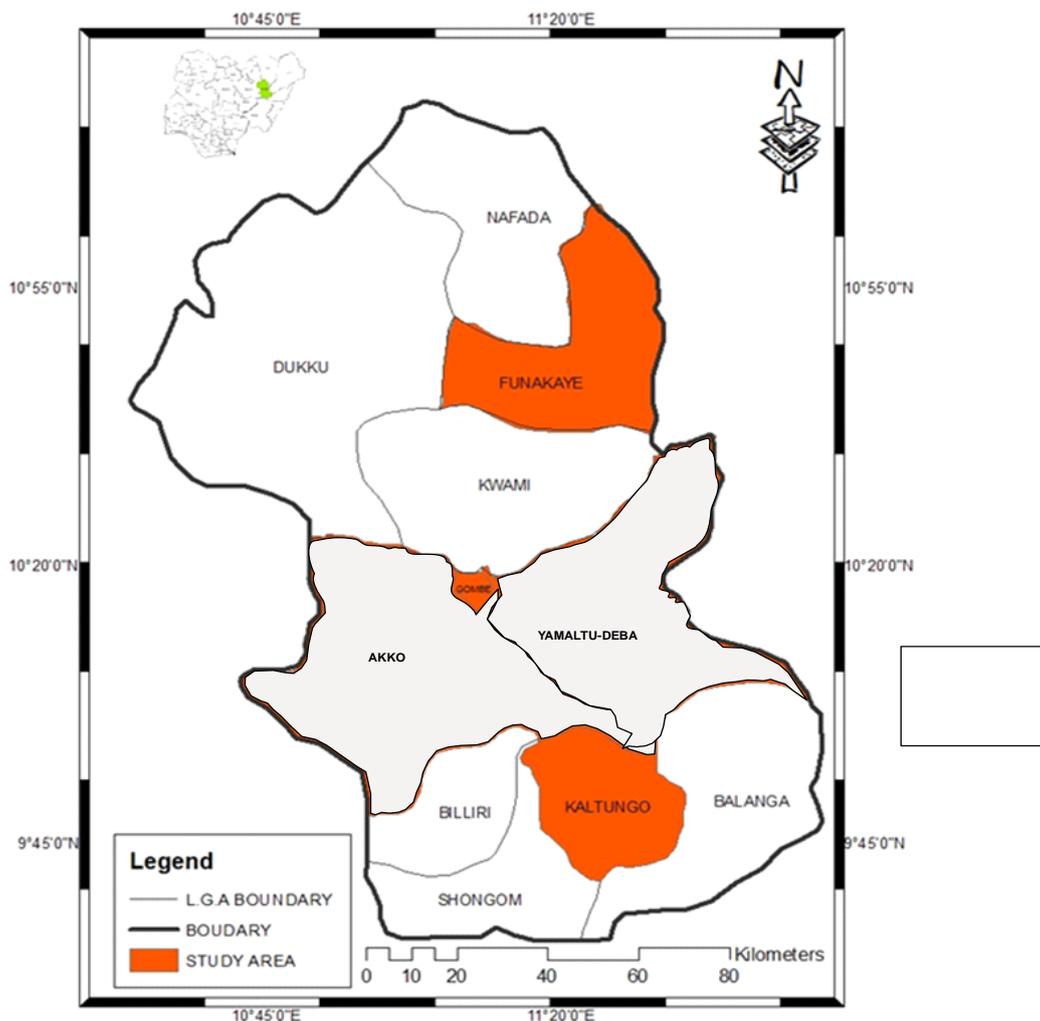


Figure 1: Maps of Nigeria and Gombe State showing the study

### Sampling and Analysis

Samples were collected from on-farm three Local Government Areas of Gombe State Nigeria namely, namely Funakaye, Gombe and Kaltungo from the three Senatorial Districts of Gombe North, Gombe Central and Gombe South respectively. The sampling technique employed was randomized sampling at every location. A homogenized sample of each of maize (*Zea mays*), millet (*Pennisetum glaucum*), rice (*Oryza sativa*), and sorghum (*Sorghum bicolor*) and beans (*Phaseolus vulgaris*) were taken from each sample locations of the Senatorial Districts. Total of 15 samples, 5g each were separated from earthly impurities, dried in an oven at 35° for 12 hours (Gomes *et al.*, 2015) and homogenized in a blender, sieved in a 2 mm sieve (Olga. 2019). Replicate amount of 100mg each of the samples were extracted using 10ml acidified acetonitrile-water (50:50) (v/v) with 1% Formic acid (v/v) in an ultrasonic bath at an ultrasonic frequency of 42 kHz for 30 minutes in a 15ml centrifuge tube (Gomes, *et al.*, 2015 and Olga, 2019). The replicates samples were then centrifuged at 4000 rpm for 10 minutes at 20°C and filtered in a Millex HV 0.45 µm with a membrane Dura pore 13 mm (Vinatoru, 2001) for Gas Chromatography and Mass Spectrophotometer (GC/MS) following the methods described in Yasushi, *et al.*, (2003).

### Health Risk Assessments

The risks assessments as described in Bwatanglang *et al.*, (2019) was conducted by evaluating the Target Hazard Quotient (THQ) to determine the potential non-carcinogenic effect of the glyphosate in the grain samples to the public on consumption. The THQ was determined by relating the estimated Average Daily Intake (ADI) for each crop samples with the Reference dose, RfD or Chronic reference dose for glyphosate (1.75 mg/kg/day) as expressed in equation (1). The ADI was estimated using equation (2) and the Health Index (HI) which is expressed as the sum of the THQ to describe the cumulative non-carcinogenic effect were expressed in equation (3). The theoretical representation of the expression in the equation (1 & 3) suggest no associate risk if the THQ or HI < 1, meaning that the exposed population is unlikely to experience any adverse health hazard and level of concern exists if the THQ or HI > 1 (Bwatanglang, *et al.*, 2019, Yaya *et al.*, 2017).

$$\text{Target Hazard Quotient (THQ)} = \frac{\text{AverageDailyDose (mgperkperday)}}{\text{ReferenceDose (Rfd)(mgperkperday)}} = \frac{ADD}{RfD} \dots\dots\dots (1)$$

$$ADI_{inh} = \frac{Cropxin \ hRxEFxED}{BW \times AT} \dots\dots\dots (2)$$

$$\text{Health Index (HI)} = \sum THQ \dots\dots\dots (3)$$

The probability of developing any type of cancer over a lifetime is calculated by integrating the CDI with the cancer slope factor (CSF) for glyphosate which is represented in the equation 4 and their respective parameters in Table 1.

$$\text{Cancer risk} = \frac{\text{Exposeddose} \times \text{riskfactor (cancer (oral)slopefactor} \times \text{yearsofexposure)}}{70 \text{ years (lifetime)}} \dots\dots\dots (4)$$

The CRI is considered negligible if the CRI < 10<sup>-6</sup>, acceptable or tolerable if CRI is 10<sup>-6</sup> < CRI < 10<sup>-4</sup>, and similarly considered high if the CRI > 10<sup>-4</sup> (Bwatanglang, *et al.*, 2019; Yaya, *et al.*, 2017).

**Table 1: Health Risk Assessment Exposure Factors: Ingestion Pathway**

Parameters	Description	Exposure (Children)	Exposure (Adults)	Reference
ED (year)	Exposure Duration	6	24	( Hu, <i>et al.</i> , 2012)
EF (days/year)	Exposure Frequency	180	180	(Liu, <i>et al.</i> , 2018)
CF (kg/mg)	Conversion factor	1.0 <sup>-6</sup>	1.0 <sup>-6</sup>	(Bwatanglang, <i>et al.</i> , 2019)
AT (day)	Non-carcinogens	ED X 365	ED X 365	(USEPA, 1989)
	Carcinogens	70 x 365	70 x 365	(USEPA, 1989)
IR <sub>ing</sub> (mg/day)	Ingestion rate	200	100	(Peng <i>et al.</i> , 2017)
BW (kg)	Body Weight	15	60	(Bwatanglang, <i>et al.</i> , 2019)

The cancer risk levels based on the USA EPA DEEM estimated dietary and drinking water exposure to glyphosate placed the average exposure of glyphosate to the general population to at 0.088 mg/kg bw/day from an estimate range of 0.058 – 0.23 mg/kg

bw/day (Solomon, 2016). The upper range of the dietary exposure cancer risk level are determined using the equating in 5 and the model estimate presented in Table 2 (Payne-Sturges, 2004).

$$\text{Cancer risk} = \frac{0.23 \frac{\text{mg}}{\text{kg}} \text{ per day} \times 0.00169 \frac{\text{mg}}{\text{kg}} \text{ per day} \times 70 \text{ years}}{70 \text{ years (lifetime)}} = 3.9 \times 10^{-4} \dots\dots\dots (5)$$

**Table 2: DEEM Model of Estimated Exposure to Glyphosate**

Exposure	Dietary Residues and Drinking Water Dose	Cancer Risk Level
Low	0.058 mg/kg bw/day	$9.8 \times 10^{-5}$
Averaged	0.088 mg/kg bw/day	$1.5 \times 10^{-4}$
High	0.23 mg/kg bw/day	$3.9 \times 10^{-4}$

**Statistical Tools:**

Analysis of Variance (ANOVA), t-Test and Pearson Product-Moment Correlation Coefficient (PPMCC) at level of significance of 5% was used for the analysis.

**III. RESULTS AND DISCUSSIONS**

**Concentrations of Glyphosate Residue in the Grains**

As shown in Table 1, the highest concentrations of glyphosate residues in the grains were detected in samples from Gombe, ranging from 0.0245ppm in maize, 0.02556ppm in rice, to 0.0548ppm in beans and 0.06615ppm in millet. However, sorghum from the results were observed to garner a low amount of 0.0136ppm in the same location. The average concentrations by sample locations were observed to follow the increasing order; Gombe > Kaltungo > Funakaye. The high glyphosate residues detected in Gombe could be traced to the high density of elite farmers that relied on agrochemicals to boost production outputs without recourse to its health-related implications. The low level of awareness on the negative effects of pesticides on human health and the environment account and hence its residual cocntration in in food crops for this high level detected in the study locations (Ogah, 2012). Modibbo and Aminu (2018) in their study reported 80% of fertilizers and 67% of herbicides applications by farmers from the Central Senatorial District of Gombe State. Bawa, *et al.*, (2015) posited among others that increased pesticide use, poor notification system and poor handling practices among others resulted in the pesticides poisoning outbreak in Gombe between 2005 and 2015. According to Chikamso (2018), illiterate farmers do not follow precautions for usage and application of pesticides thereby increasing the risk of exposure to human and the environment.

In the same token, the duo of beans and rice occupy prominent positions in the hierarchy of staple food in the Northern region. Nigeria is the largest producer of beans in Africa with about 500,000 metric tonnes. Beans is a legume useful as oil, animal feeds, soya milk and cake called ‘‘awara’’ in the North. Beans from Gombe (0.0548ppm) followed by Kaltungo beans (0.03049ppm) with a distant value of 0.01604ppm from Funakaye. Rice is in the order of Funakaye > Gombe > Kaltungo this may be attributed to extent of the suitability of the soil for the crop and size of farmers` involvement from North to Southern part of Gombe State. The lowest concentrations of the residues range from below detectable limit value of millet from Funakaye, Funakaye maize (0.00688ppm) to Kaltungo millet (0.00873ppm), Gombe sorghum (0.01415ppm), Gombe sorghum (0.0136ppm), Funakaye sorghum (0.0169ppm), beans (0.0169ppm), Kaltungo maize (0.0173ppm) and rice (0.01746ppm) respectively. The reasons for the low concentrations of residues in these areas are as submitted above. These are feeder Local Government Areas around Gombe town that are not as populated as Gombe Local Government Area. They may be more endowed with better arable land and more fertile soils, but readily available manpower to be engaged in weed clearing and other activities is better than the Gombe situation. The soil type may as well result in the low level residues discovered in most parts of the study area. Conditions that favor movement of the herbicide into soil solution tend to increase absorption by plants. Rainfall is scanty and short-lived in most part of the study area. Moreover, the study area is generally prone to soil erosion and surface run-off that usually unsettle the herbicide after application. Glyphosate, being a phosphonic amino derivative of glycine tends to behave like inorganic phosphate naturally occurring in the soil. A large portion of the herbicide present in soil is bound to soil colloids (clay, organic matters) and this herbicide is less readily available to the plants than the one present in soil solution. Strong sorption to most soils, low mobility, and low tendency to leach and migrate into groundwater, low volatility and resistant to chemical degradation results in

slow dissipation of glyphosate (TPG, 2019, Vincenzor, *et al.*, 2018). However, as shown in the table, the results revealed that the concentrations of glyphosate residues in the grains are below the Maximum Residual Limit (MRL) set by the WHO (1996).

**Table 1: Average glyphosate concentration according to the Local Government Areas, average recovery and Maximum Residual Limit (MRL)**

	Maize (ppm)	Beans (ppm)	Sorghum (ppm)	Millet (ppm)	Rice (ppm)
Funakaye	0.00688	0.01639	0.0169	*BDL	0.0305
Gombe	0.0245	0.0548	0.0136	0.06615	0.02557
Kaltungo	0.0173	0.03049	0.01415	0.00873	0.01746
%AR±S. Error	74.53±0.36	75.10±1.81	82.43±0.45	86.17±0.67	93.47±1.22
MRL (WHO/FAO)	5.0	*N.D	30	30	*N.D

\*BDL (Below Detection Limit) \*ND (Not Detected), %AR (Percentage Average Recovery)

### Health Risk Analysis

The average daily intake ADI of glyphosate as presented in Table 2 on the consumption of the grains used for the estimation of THQ and the HI obviously shows a much lower value than the 0.5mg/kg standard presented by the European Food and Safety Authority (EFSA), except in the case of beans, with ADI value of 0.546mg/kg/day for children. The ADI results for the children in three out of the five categories of grains are greater than those of the adults. The ADI for maize, beans and millet in particular are greater than their adult counterparts. Indicated that the children in the study locations are at greater risks and more susceptible to the exposure of glyphosate via the ingestion of glyphosate-contaminated food. The results show that the HI for the children is greater than that of the adult; indicating once again that children are more susceptible than the adults. The children will be more likely at risk than adults in the study area. However, none of the results either equals  $\geq 1$ . Since both  $HI < 1$ , the concentrations in the grains do not pose any significant risk on the target population (Yaya, *et al.*, 2017; and Bwatanglang, *et al.*, 2019). The HI for both the children (6.47E-01) and adult (4.67E-02) are practically less than 1 and therefore, are not at any risk of adverse health challenge. However, the HI for the children is too close to 1 for comfort. The residues have a chance of accumulation over repeated use and over time, the resultant effect on human health and the environment can result to pose risk.

Furthermore, the lifetime cancer risk (CRI) estimated for the children and adult as presented in the Table 2, in all the cases are high, but still within the acceptable range of  $10^{-6}$  -  $10^{-4}$  (Bwatanglang, *et al.*, 2019, Yaya, *et al.*, 2017). In accordance with the USA EPA Dietary Exposure Evaluation Model (DEEM) CRIs are ranked as Low Risk when values are within  $9.8 \times 10^{-5}$ , Average Risk  $1.5 \times 10^{-4}$  and High Risk for  $3.9 \times 10^{-4}$  respectively (Pagne-Sturges, 2004; Solomon, 2016). The results of CRI presented above could be safely adjudged but however discomfoting. This result portends potential danger and calls for proactive measures and regulation on the use of the herbicides. In general, the CRI result is observed to be higher in children than adult as the Total Cancer Risk Index (TCRI) shows  $1.81 \times 10^{-3}$  and  $2.26 \times 10^{-4}$  for children and adult respectively.

**Table 2: Carcinogenic and non-carcinogenic characterization of glyphosate in the different grain samples by ingestion pathway for both children and adults. Cancer Risk Index, CRI in Bracket**

	THQ (Children) Ingestion Route	Cancer Risk Index (CRI) (Children)	THQ (Adults) Ingestion Route	Cancer Risk Index (CRI) (Adults)
Maize	0.06	$1.80 \times 10^{-4}$	0.0076	$2.25 \times 10^{-5}$
Sorghum	0.06	$1.66 \times 10^{-4}$	0.0079	$2.09 \times 10^{-5}$
Beans	0.31	$9.23 \times 10^{-4}$	0.0039	$1.15 \times 10^{-5}$
Rice	0.13	$3.87 \times 10^{-4}$	0.016	$4.83 \times 10^{-5}$
Millet	0.08	$1.49 \times 10^{-4}$	0.011	$1.86 \times 10^{-5}$
HI ( $\Sigma$ THQ)	<b>0.65</b>	<b>TCRI (<math>1.81 \times 10^{-3}</math>)</b>	<b>0.0467</b>	<b>TCRI (<math>2.26 \times 10^{-4}</math>)</b>

### Statistical Comparative Analyses

Tables 3-7 present the results of statistical analyses. Tables 3-5 gave the results of t-Tests between pairs of the glyphosate residues in the grains sampled from the LGAs at level of significance of 5%. The t-Test in Tables 3 and 4 for Funakaye and Gombe and between Kaltungo and Gombe both rejected the null hypothesis, indicated significance difference the residues in the grains as made obvious in Table 1. The results of residue concentrations in the grain samples from Gombe stand out among the

three locations. However, the t-Test result between Funakaye and Kaltungo accepted the null hypothesis; that there is no significant difference between them. In Table 6, the ANOVA result shows that there is no significant difference among the LGAs under study. In Table 7, the results of the Pearson Product-Moment Correlation Coefficient produced results that accepted the null hypothesis that there is no significant correlation between the LGAs. This may be due to the fact of the peculiarity of each of the LGAs under review as already explained under glyphosate residues concentrations above.

**Table 3: Results of t-Test analysis to determine the statistical significance difference between Funakaye and Gombe Local Government Areas**

L.G.A	N	Mean	Variance S <sup>2</sup>	Standard Dev.	df	α	t-cal	t-critical	Decision
Funakaye	5	0.014	1.28 x 10 <sup>-4</sup>	0.11	8	0.05	-1.96	1.86	H0 Rejected
Gombe		0.037	5.03 10 <sup>-4</sup>	0.02					

*Null Hypothesis (H0): There is no significant difference in the concentration of glyphosate between grain samples from Funakaye and Gombe Local Government Areas.*

**Table 4: Results of t-Test analysis to determine the statistical significance difference between Funakaye and Kaltungo Local Government Areas**

L.G.A	N	Mean	Variance S <sup>2</sup>	Standard Dev.	df	α	t-cal	t-critical	Decision
Funakaye	5	0.014	1.28 x 10 <sup>-4</sup>	0.11	8	0.05	-0.16	1.86	H0 Accepted
Kaltungo		0.015	1.18 x 10 <sup>-4</sup>	0.01					

*Null Hypothesis: There is no significant difference in the concentration of glyphosate between grain samples from Funakaye and Kaltungo Local Government Areas.*

**Table 5: Results of t-Test analysis to determine the statistical significance difference between Gombe and Kaltungo Local Government Areas**

L.G.A	N	Mean	Variance S <sup>2</sup>	Standard Dev.	df	α	t-cal	t-critical	Decision
Kaltungo	5	0.0151	1.18 x 10 <sup>-4</sup>	0.11	8	0.05	1.95	1.86	H0 Rejected
Gombe		0.0369	5.03 x 10 <sup>-4</sup>	0.02					

*Null Hypothesis: There is no significant difference in the concentration of glyphosate between grain samples from Kaltungo and Gombe Local Government Areas.*

**Table 6: Analysis of Variance (ANOVA) of the concentrations of glyphosate in grain samples from the different Local Government Areas under study**

Sources of Variance	Df	Sum of Squares (SS)	Mean Squares	F-Cal	F-Critical	Decision
Between Groups	(K-1) 2	3.35 x 10 <sup>-4</sup>	1.68 x 10 <sup>-4</sup>	0.21	3.89	Accepted Ho
Within Groups	(N-1) 12	9.8 x 10 <sup>-4</sup>	8.17 x 10 <sup>-4</sup>			
Total	14	1.02 x 10 <sup>-4</sup>				

*Null Hypothesis (H0): There is no significant difference between the levels of concentrations of glyphosate residues in the grain samples from 3 Local Government Areas.*

Table 7: Results of Correlation Coefficient Analysis of the level of glyphosate residues in the grains from the Local Government Areas

LGA	N	Cal. r	Critical r	Level of sigf. ( $\alpha$ )	Degree of Coeff.	of Df	Decision
Fun/Gombe	5	-0.52	0.88	0.05	ve-,High	3	H0 Accepted
Fun/Kalt		0.21			ve+, Low		H0 Accepted
Kalt/Gombe		0.37			ve+, Moderate		H0 Accepted

Note: Fun = Funakaye, Kalt = Kaltungo. Null Hypothesis: There is no significant correlation between in glyphosate residues in the sample of grains in the pairs of Local Government Areas.

#### IV. CONCLUSION

The exposed population under study, by the above results is under serious threat of being infected one way or the other by life-threatening diseases from the continual use and consumption of glyphosate impregnated food crops in the future. This therefore, calls for concerted efforts by environmentalist, government and non-governments bodies, civil society groups to rise to the occasion by taking calculated steps in mitigating the indiscriminate use if total avoidance of glyphosate-based herbicides is impracticable to preserve nature and safe humanity from this self-inflicted imminent carnage.

**FUNDING:** This research was funded by Tertiary Education Trust Fund, Nigeria (TETFUND)

**ACKNOWLEDGMENTS:** The authors wish to acknowledge the Department of Pure & Applied Chemistry, Faculty of Science, Adamawa State University Mubi and Tertiary Education Trust Fund (TETFUND), Nigeria.

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**Citation of this Article:**

Zira, S. P., Bwatanglang, I. B., & Audu, Haruna Sympa. (2024). Exposure Assessment of Glyphosate in Selected Grains Grown in Gombe State, Nigeria and the Potential Health Risk on Consumption. *International Research Journal of Innovations in Engineering and Technology - IRJIET*, 8(10), 105-113. Article DOI <https://doi.org/10.47001/IRJIET/2024.810016>

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